“Techniques in Cellular and Molecular Neurobiology”

International Master’s Degree in Neuroscience

Lesson 3

DIPARTIMENTO DI SCIENZE DELLA VITA

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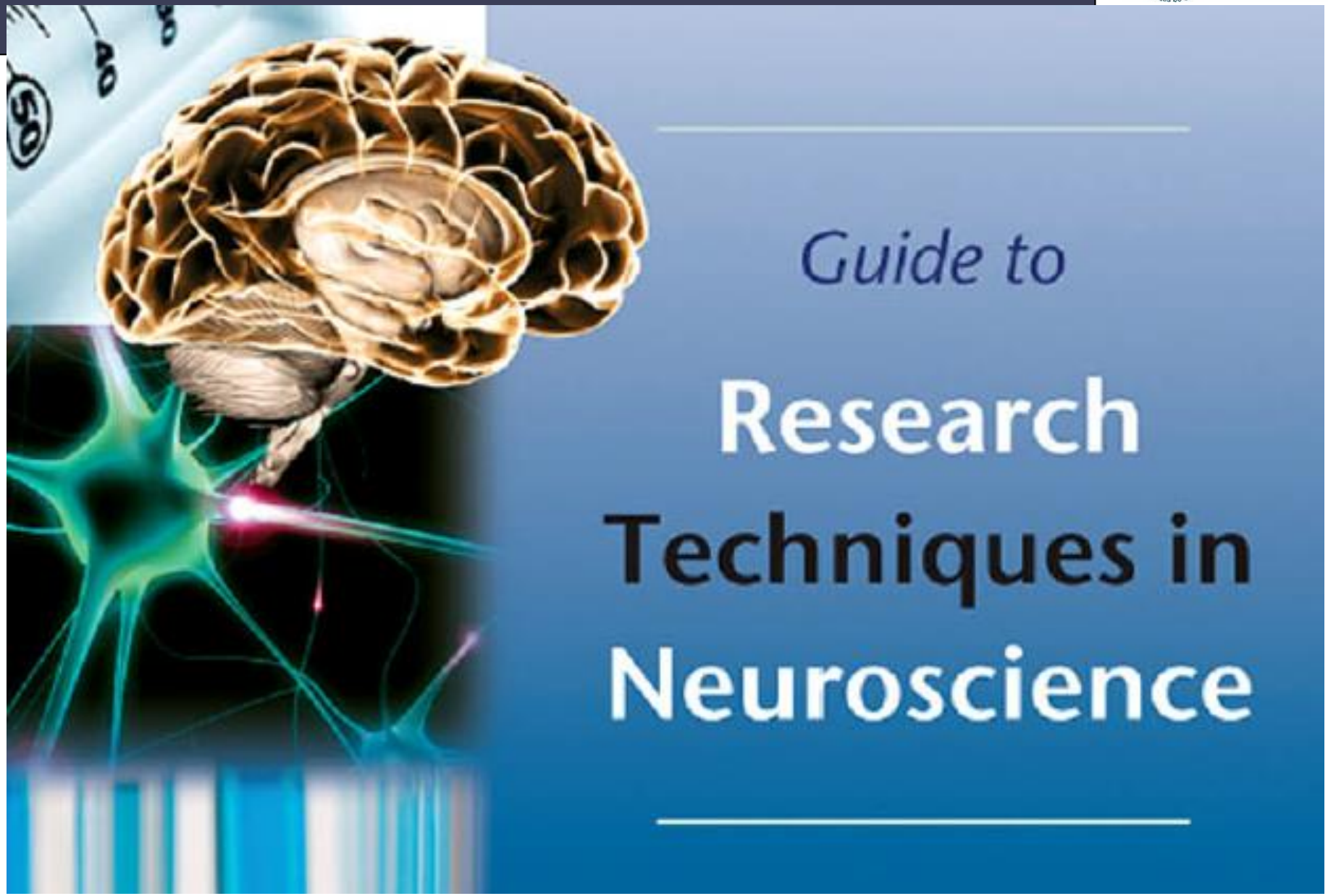

Cell Culture techniques

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Cell Culture techniques

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After reading this chapter, you should be able to:

- Explain the advantages and disadvantages of using *in vitro* culture techniques
- Compare types of cells used to examine nervous system function *in vitro*
- Describe techniques for manipulating *in vitro* cultures

Examining the nervous system *in vitro* (“ within glass ”) allows scientists to simplify the cellular environment, providing greater control over experimental manipulations and reducing potentially confounding interactions with other biological systems.

Cell Culture techniques

- *In vitro* tools and techniques make experiments possible that would otherwise be difficult or impossible to perform (or interpret) in intact organisms, such as performing multiple assays in parallel with the exact same number of cells.
- Reducing the complexity of the experimental preparation, *in vitro* experiments tend to be faster, less expensive, and require fewer animals than experiments performed *in vivo.*

Cell Culture techniques

Specialized equipment and reagents are necessary to provide cultured cells with an environment that can support their continued growth and health outside a living organism.

- Most of these supplies are used to artificially mimic the endogenous, *in vivo,* cellular environment.
- Other tools and reagents prevent contamination. Cells in vivo have the benefit of an active immune system to prevent contamination from bacteria,fungi, and other microorganisms.

Fundamental pieces of equipment

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- **Biosafety Hood.** A biosafety hood or laminar flow cabinet is used to prevent contamination by microorganisms. When not in use, these chambers are often illuminated with UV light that helps sterilize exposed surfaces.
- **Cell Incubator.** Cell incubators house and store culture flasks/plates. These incubators maintain an appropriate temperature, humidity, and gas concentration to mimic endogenous conditions. They are usually set at 37 ° C with 5% CO 2 levels.
- **Centrifuge.** Cell often require a change of the media and the possibility to separate cells from media when in suspension represent an essential asset**.**
- **Treated cell culture flasks/plates.** Tissue culture flasks and plates come in many varieties depending on the needs of the investigator.

Fundamental pieces of equipment

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- **Refrigerator.** A refrigerator (properly referred to as a 4 ° C incubator) maintains cell culture media and other reagents when not in use.
- **Water bath.** A water bath is often set at 37 ° C and is used to quickly warm cell culture media and other reagents stored at 4 ° C.
- **Microscope.** Microscopes are used in most tissue culture rooms for routine observation of cell culture flasks/plates to inspect the health and confluence of cells.

Culture Media

- Growth media is critical to cell culture experiments, supplying nutrients (amino acids and vitamins) and a source of energy (glucose) for cells. Growth media can vary in pH, nutrient concentration, and the presence of growth factors or other biologically relevant components. To survive, cells must be bathed in an isotonic fluid that has the same concentration of solute molecules as inside the cell. The media is buffered to maintain a compatible pH (usually 7.4, though there are some cell-specific variations).
- Serum is often added to culture media for its ability to promote survival through undefined mixtures of growth factors, hormones, and proteins, like PDGF (platelet-derived growth factor), insulin, and transferrin.
- For stricter control over the cellular environment, investigators use serum-free, chemically defined supplements, such as N2 or B27, that contain known formulations of survival factors.

CELL LINES

Advantages	Disadvantages
Controlled physiochemical environment (pH, temperature, oxygen, carbon dioxide, osmotic pressure, etc.)	Unstable aneuploid/polyploid chromosome constitution
Controlled and defined physiological conditions (constitution of medium, etc.)	They might not have the relevant attributes or functions of relatively normal cells.
Homogeneity of cell types (achieved through serial passages) and “easy” to manipulate gene expression	They express unique gene patterns not found in any cell type in vivo
Economical, since smaller quantities of reagents are needed than in vitro (1/10)	

- Cell lines / strains (transformed cells)

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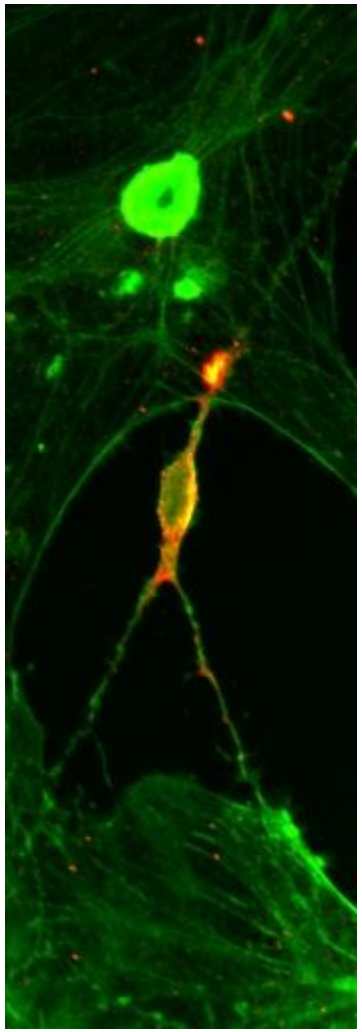
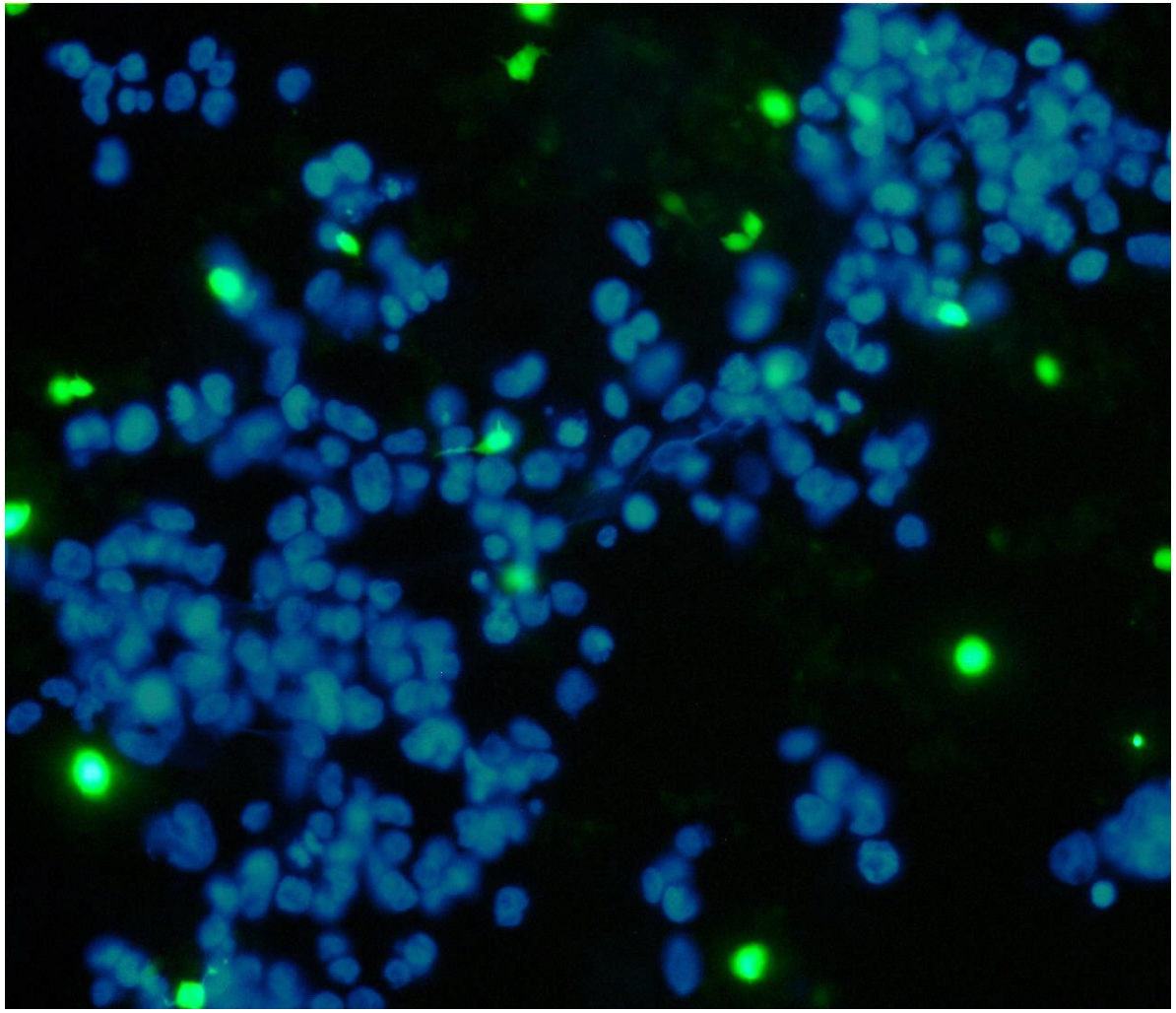
Primary cells



long term cell line



- Cell lines / strains (transformed cells)

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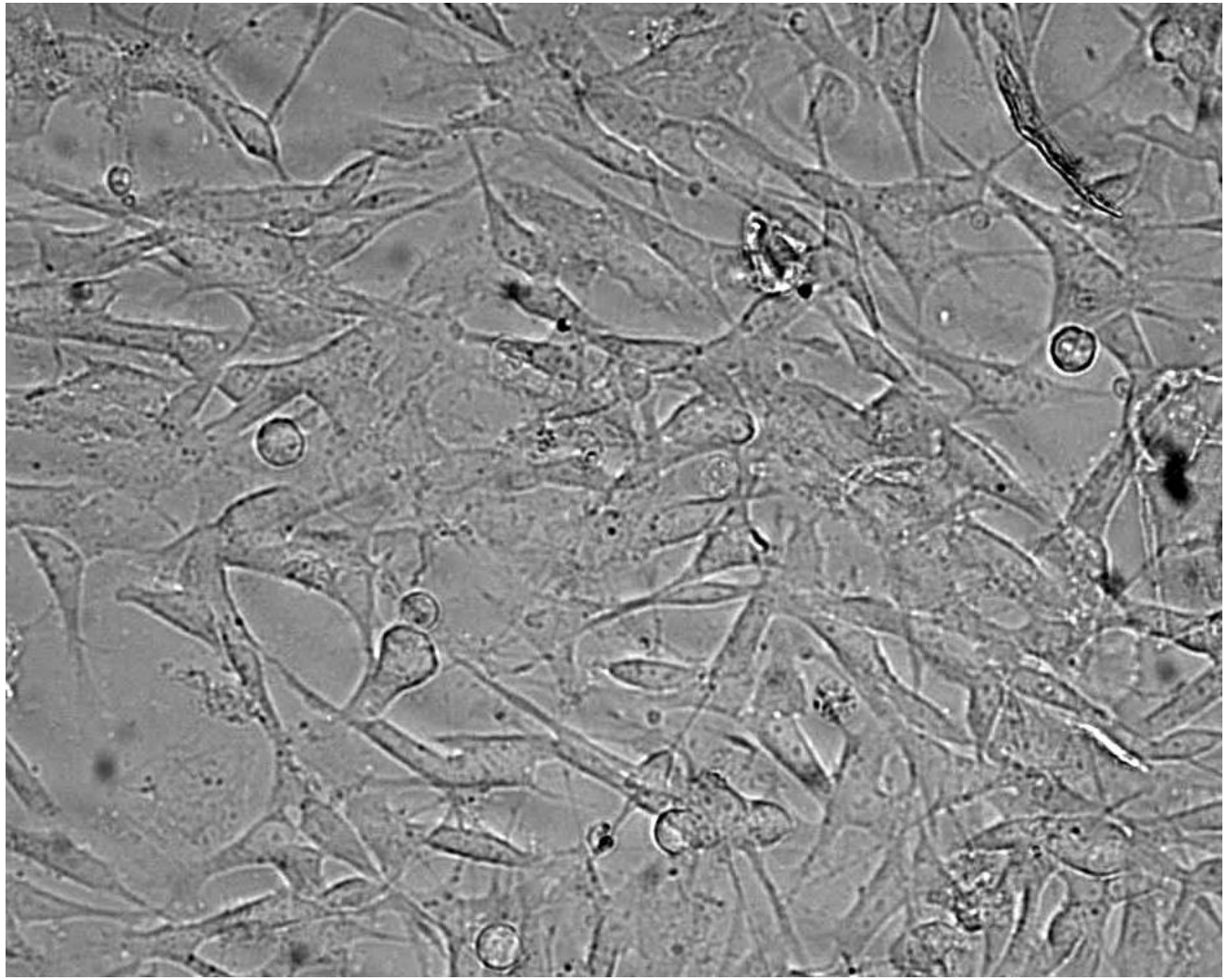
Protein

1. Western BLOT (limitation due to strain)
2. Immunocito (limitation due to strain)
3. ELISA
4. Overexpression
5. Downregulation

- Cell lines / strains (transformed cells)



• Cell lines / strains (transformed cells)

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RNA

1.PCR

2.Real Time-PCR

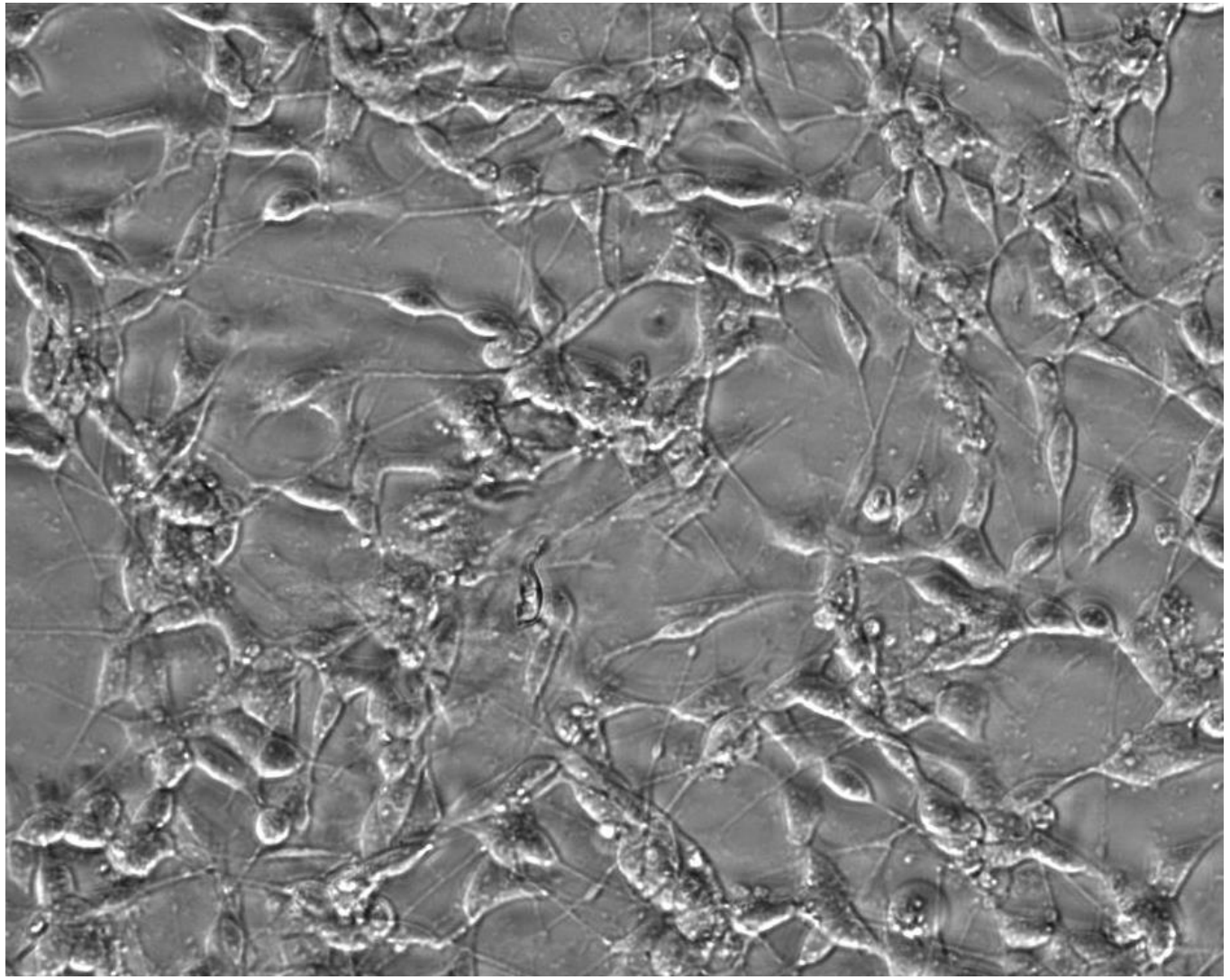
3.Northern Blotting

4.InSitu Hyb

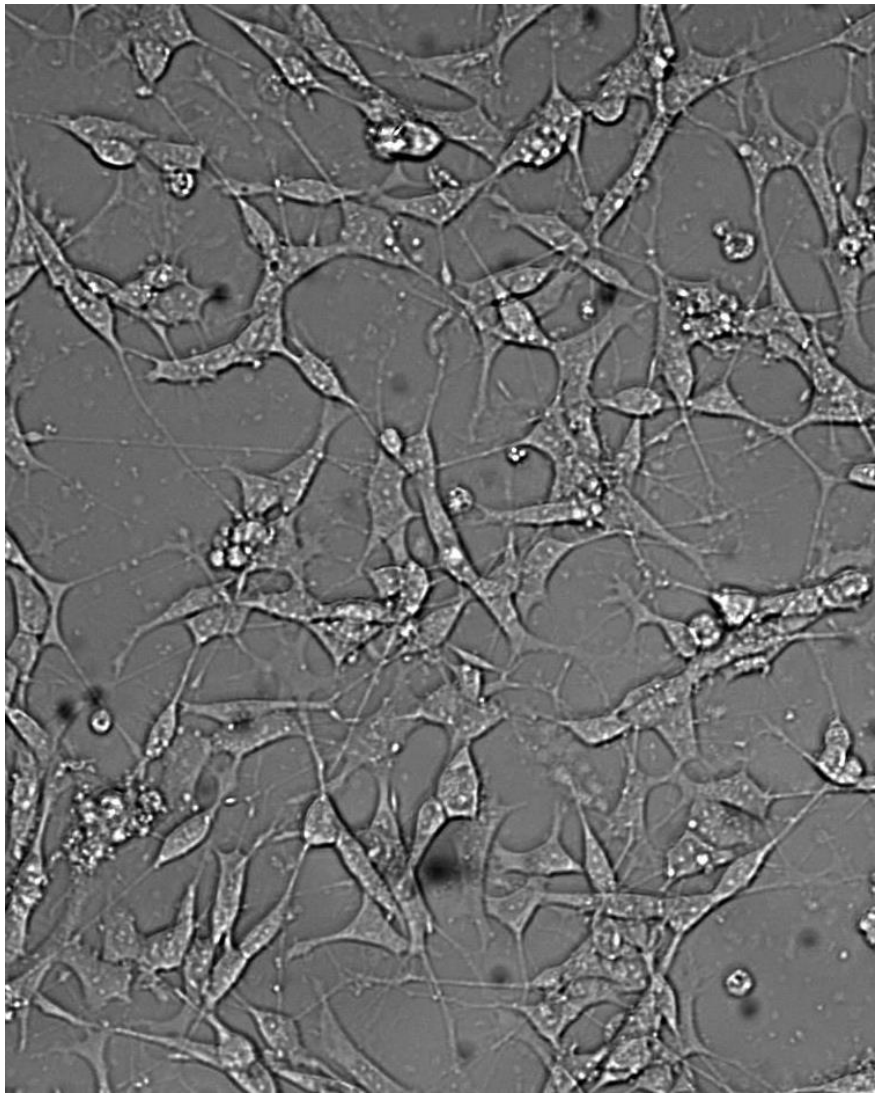
5. Translatability assay (ad es. Luciferase)

Electrophysiology
generally not used

- Cell lines / strains (transformed cells)



- Cell lines / strains (transformed cells)



Cell lines VIDEO 1

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Cell lines VIDEO 2

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Cell lines VIDEO 3

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Cell lines VIDEO 4

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Primary cells

- **Primary cell and tissue culture** uses tissue removed directly from a living animal rather than immortalized cells that continuously divide in a dish.

- There are three main categories of primary tissue culture: dissociated cultures, explants (used just in acute), and slice cultures.

PRIMARY CELLS : preparation

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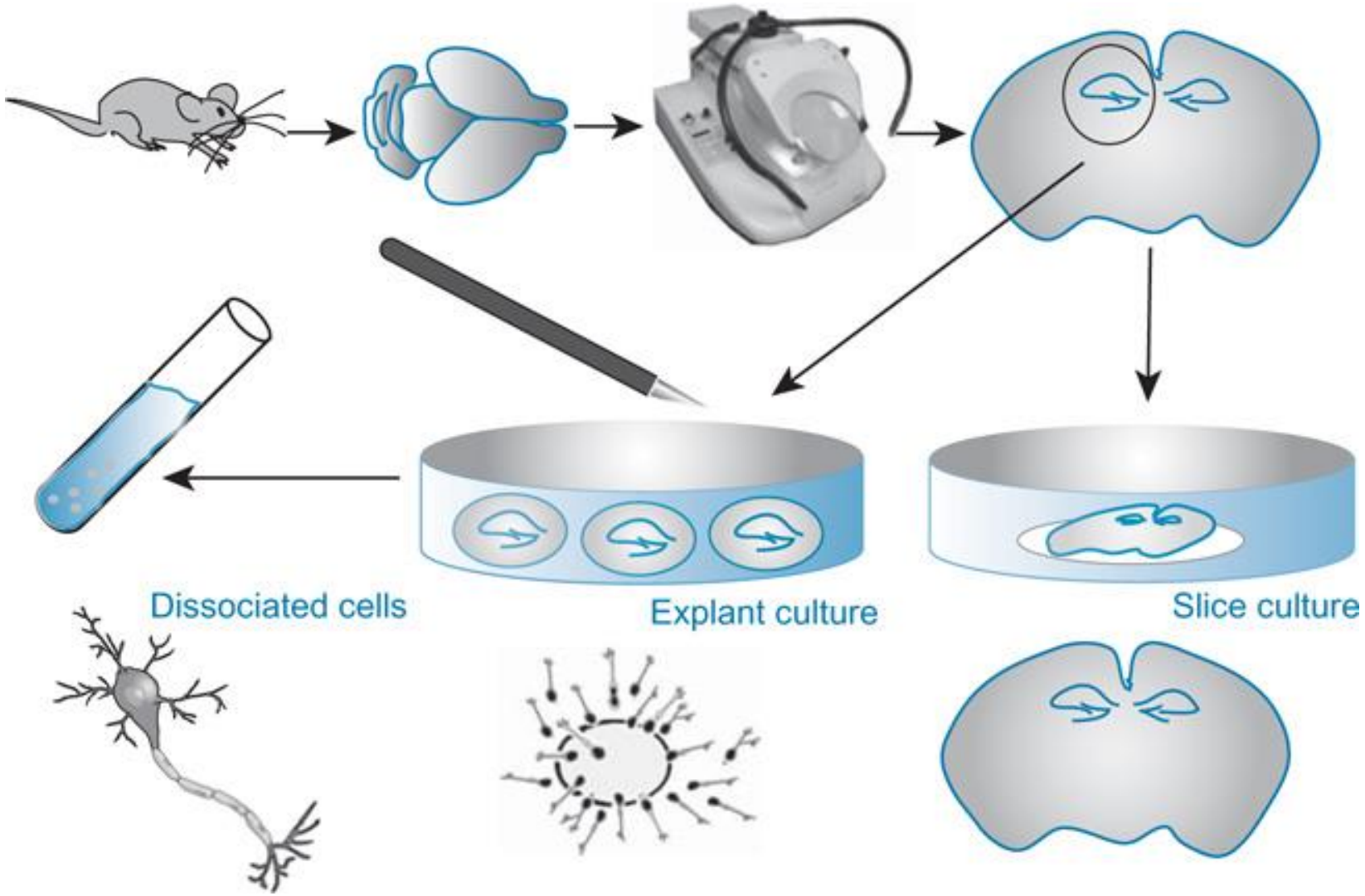

PRIMARY CELLS

- Neurons dissociated from different regions of the brain retain their initial identities.
- The morphological, molecular, and physiological properties of cell populations present in culture correspond closely to the characteristics of the cell population present in the region of origin in a living organism.
- With the proper growth factors and care, it is possible to maintain a dissociated culture for weeks, during which time cells acquire properties of mature neurons.
- They develop characteristic axons and dendrites, form synapses with one another, and express receptors and ion channels specific to their cell types, even producing spontaneous electrical activity.

PRIMARY CELLS

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- Dissociated neuronal cell cultures have been used to study neurite outgrowth, synapse formation, and electrophysiological properties.
- The ability to probe individual neurons, however, comes at the expense of losing the organization and connectivity critical to *in vivo* functions.
- Limitation of dissociated cultures is the small quantity of material produced,which can make biochemical analyses more difficult

PRIMARY CELLS : ADVANTAGES

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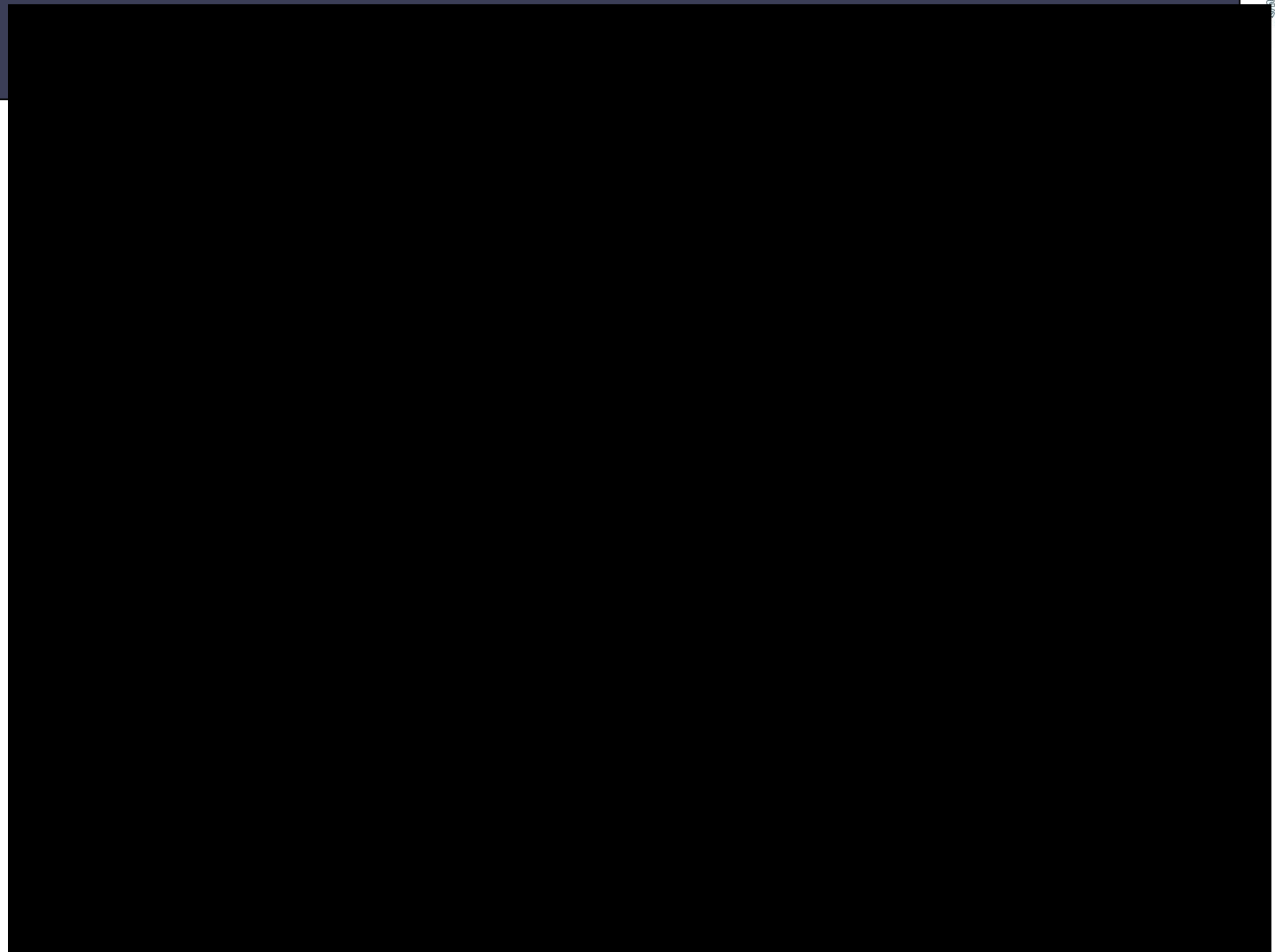
- Primary tissue culture allows scientists to directly investigate cells of interest, probing the functions of specific neuronal or glial subtypes and the cellular and molecular dynamics specific to certain cell types
- Examining neural tissue in vitro is better for recording electrophysiological properties, visualizing dynamic structure and function, and using pharmacology to manipulate function.
- By examining tissue cultured from genetically manipulated organisms, scientists can observe the cellular and molecular effects of the genetic modification.
- Primary tissue culture allows investigators to more confidently extrapolate results directly to the intact nervous system.

PRIMARY CELLS : DISADVANTAGES

- Primary cultures have a limited lifetime, unlike immortalized cell lines. Moreover neurons do not duplicate , therefore limited amount of material.
- Primary cultures are more difficult to manipulate genetically to modify gene expression
- The age of the animal source influences the health and robustness of the cell culture: tissue from younger, embryonic or early postnatal animals survives better and tends to be healthier than tissue from older animals.
- A population of primary cells will always be more heterogeneous than a culture of immortalized cells, no matter how careful the scientist was in extracting and purifying the cells of interest.

Primary Cells VIDEO 1



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